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Art. XV.—*Some Algae of Victorian Soils.*

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Although for a long time it has been recognized that many algae, especially Myxophyceae, grow on the surface of the soil, it is only during comparatively recent years that it has been realized to what a large extent they inhabit the deeper layers of the soil, and what a significant part they play in its economy. Their exact role has not yet been determined, but Schramm(34) and later Wann(42) considered that they had the power of fixing free nitrogen. Moore and Webster(23) also claimed that they had obtained definite nitrogen fixation by algae, but as their cultures were not free from bacteria, this may have affected their results. This theory was not upheld by Bristol-Roach and Page(9). Waksman(40), however, suggests that algae may aid bacteria in the fixation of nitrogen by providing the carbohydrates required by the bacteria. Whatever their part, their abundance in the soil suggests that they cannot be without some effect on its fertility.

Algae are more abundant in the upper layers of the soil, but they may exist at depths of 100 cm. beneath the surface. They are more numerous in manured than in unmanured ground. No very satisfactory count of their numbers has been made, but according to Bristol-Roach(4), they vary from 700 to many thousands per gram according to the conditions of the soil.

These soil Algae belong to four groups; (i) Myxophyceae or Blue-green Algae, (ii) Chlorophyceae or Green Algae, (iii) Heterokontae, (iv) Bacillariaceae including Diatoms. The Chlorophyceae and Heterokontae are universally distributed, while the Myxophyceae appear as a general rule in greater numbers in cultivated rather than in uncultivated soils(4), and Diatoms appear mostly in soil from old gardens(40).

In England, America, Germany, and Africa, soil microbiologists have succeeded in identifying soil forms representing over a hundred genera. However, no work of this kind has previously been attempted in Victoria or even, for that matter, in Australia. There have been, however, some investigations in connexion with fresh-water forms in Australia and New Zealand. As far

as Victoria is concerned the most outstanding work is that of West(43) on the algae of the Yan Yean Reservoir. In this work, Professor West has identified over 300 species from material forwarded to him by Mr. Hardy of the Lands Department, Melbourne.

The purpose of this investigation is to identify as many Victorian soil species as possible. At first it was intended to make a count of the number of cells of each species per gram of soil using the method suggested by Bristol-Roach(6), but this was found to be impossible until the forms could be identified with greater ease. It is first necessary to obtain cultures of the organisms, and since many algae exhibit polymorphism in a marked degree, it is preferable to isolate each species and obtain cultures which are as far as possible pure—at least free from other algae even if contaminated by bacteria or fungi. This has not been possible in all cases, but some were readily obtainable in pure culture.

The following method was used: By means of a sterilized soil auger, 10 grams of soil were obtained, passed through a sterilized 3 mm. sieve, and then placed in a bottle containing 100 c.c. of a sterilized mineral salts solution, thus giving a 1 in 10 suspension. The solution was that recommended by Bristol-Roach(6) and was of the following composition:—

KH_2PO_4	1·0 gm.
$NaNO_3$	1·0 gm.
$MgSO_4 \cdot 7H_2O$	·3 gm.
$CaCl_2$	·1 gm.
$FeCl_3$	·01 gm.
Glass-distilled water	2,000 c.c.

Glass-distilled water is essential as water distilled in an ordinary copper still is unsuitable for this work, since even minute traces of copper are toxic to many algae.

The 1 in 10 suspension was carefully shaken for half an hour in order to thoroughly separate the organisms. This prolonged shaking was necessary because of the difficulty in separating the cells of many species on account of the layer of mucilage investing them. It is because of this difficulty that it has so far been impossible to obtain a really satisfactory count of the total number of algae present in the soil, especially of the Myxophyceae. After shaking, 50 c.c. of the 1 in 10 suspension were pipetted into an erlenmeyer flask containing 50 c.c. of the sterile mineral salts solution, thus giving a 1 in 20 suspension. From this second suspension, 50 c.c. were transferred into another flask containing 50 c.c. of sterile mineral salts solution giving a 1 in 40 suspension. This process of half and half dilution was repeated until a series of suspensions was obtained ranging

in dilution from 1 in 20 to 1 in 1,310,720 in the seventeenth flask. These flasks were then exposed to light in a window with a northern aspect, the light stimulating the growth of the algae but retarding to a certain extent the development of contaminating bacteria. In four to five weeks' time, the surface of the solutions became quite green and the algae present were examined. The soil for the first and second series of cultures was taken from a garden at the Melbourne University, the soil being derived from Silurian mudstone and sandstone. The first sample was taken in July, 1930, and in the following March, a second series of flasks was set up in the above manner. Three months later, at the end of May, a third series was set up. This third sample was taken from virgin bush soil at Heathmont which is also in the Silurian belt. From these mixed cultures, pure cultures were then obtained as far as possible. There are two factors which render this task difficult; (i) the mucilage investing many forms making them not readily separable, and (ii) their slow growth in comparison with either fungi or bacteria. To obtain pure cultures the ordinary plating-out method was first used. The medium was mineral salts agar, 1.75 gm. of agar being added to a litre of the mineral salts solution previously used. This medium is suitable for the growth of algae, but is entirely unsuitable for the growth of most fungi and bacteria.

A loopful of the solution in No. 1 flask was put in 5 c.c. of sterilized mineral salts solution and carefully shaken for half an hour. From this, 1 c.c. was pipetted into a sterilized petri dish and then approximately 10 c.c. of the liquid agar medium at about 37° C. was run into the petri dish and left to solidify. This process was repeated with each flask, until a series of plates was obtained. In these plates the algae grow in colonies and can be cut out aseptically and transferred to fresh media. In this way pure cultures of *Chlorococcum humicola* (Naeg.) Rabenh. *Stichococcus bacillaris* Naeg., *Ulothrix variabilis* Kütz, *Bumillaria exilis* Klebs, *Phormidium tenue* (Menegh.) Gomont., *Phormidium autumnale* (Ag.) Gomont., *Trochiscia hirta* Hansgirg, *Protococcus viridis* Agardh, *Chlorella vulgaris* Beyerinck, have been obtained. Many organisms identified in the solutions in the flasks have not so far been obtained in pure culture. These include *Nostoc paludosun* Kütz, *Nostoc muscorum* Kütz, *Botrydiopsis arhiza* Borzi, and many others. Other organisms have not yet been identified or subcultured. The Chlorophyceae are by far the most numerous in the cultures, while very few Diatoms can be found. Only three genera of the Myxophyceae have been observed, and these did not develop for many weeks after the Chlorophyceae first appeared.

The following are the species which have been successfully identified as present in Victorian soil:—

—	*Garden Soil A.*	*Garden Soil B.*	*Bush Soil.*
I. Myxophyceae—			
Nostoc paludosum Kütz	×	×	
Nostoc muscorum Kütz	×		
Anabaena minutissima Lemm. ..	×		
Anabaena variabilis Kütz. ..			×
Phormidium foveolarum Gom. ..	×		
Phormidium tenue Gom.	×	×	
Phormidium autumnale Gom. ..	×		
Phormidium australe, n. sp. ..	×		
Phormidium subterraneum, n. sp. ..			×
II. Chlorophyceae—			
Chlamydomonas communis Snow ..			×
Chlamydomonas communis Snow., var. grandis, n. var.			×
Chlamydomonas gracilis Snow ..		×	
Chlorococcum humicola Rabenh. ..	×	×	×
Chlorella vulgaris Beyerinck. ..	×	×	×
Ourococcus bicaudatus Grobéty ..			×
Ankistrodesmus falcatus Ralfs. ..			×
Oocystis solitaria var. terrestris, n. var.	×		
Trochiscia hirta Hansgirg	×	×	
Protococcus viridis Agardh. ..	×	×	×
Muriella australis, n. sp.		×	
Ulothrix variabilis Kütz	×	×	
Ulothrix subtilissima Rabenh. ..			×
Ulothrix aequalis Kütz			×
Hormidium flaccidum A.Br. ..	×	×	×
Stichococcus bacillaris Näg. ..			×
Gongrosira australis, n. sp. ..			×
Vaucheria hamata (Vauch.) Lyngb. ..			
III. Heterokontae—			
Botrydiopsis arhiza Borzi		×	
Chlorocloster minor, n. sp.			×
Ophiocytium terrestre Heyward ..			×
Heterococcus viridis Chodat ..	×		×
Bumilleria exilis Klebs	×	×	×
IV. Bacillariaceae—			
Hantzschia amphioxys (Ehr.) Grun. ..	×	×	
Navicula mutica Kütz	×		

Myxophyceae.

The Myxophyceae do not appear in the culture flasks until some months after the Chlorophyceae, and as a rule they appear in the flasks at the beginning of the series which are less dilute. Only three genera, *Nostoc, Phormidium,* and *Anabaena* were found, though each was represented by more than one species.

The Myxophyceae are difficult to obtain in pure culture, and so far only two have been successfully grown—*Phormidium autumnale* and *P. tenue*. However, it was quite possible to identify the other species from the mixed flasks without any measure of doubt. The following are the species found, with a brief description of each:—

Nostoc paludosum Kütz.

(Figure 1.)

This species was found in flasks Nos. 1 and 2 of the culture of garden soil A. Tiny hemispherical colonies were formed on the sides of the flask slightly above the level of the liquid medium. Where the edges of two colonies met, the sides became flattened (Fig. 1. A). Upon examination, each colony was seen to be without a firm outer sheath (Fig. 1. B), but each individual

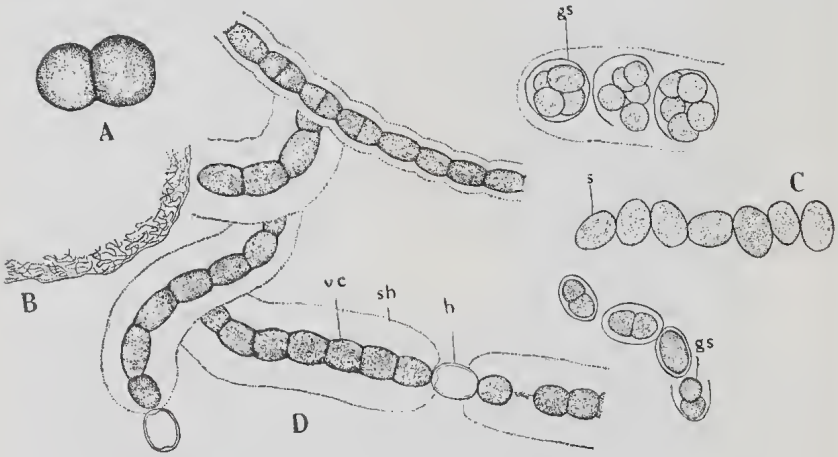


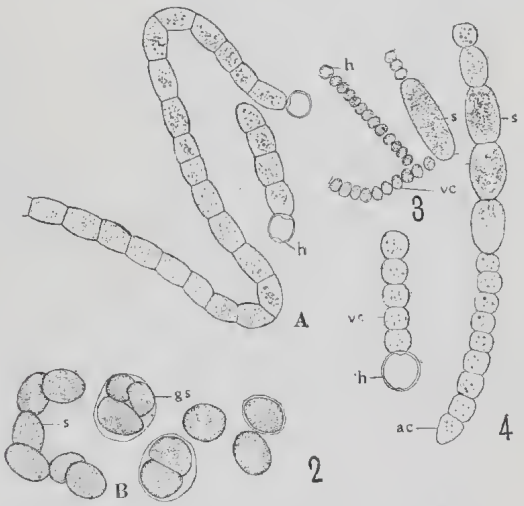
Fig. 1. Nostoc paludosum Kütz. A. Macroscopic view of two colonies side by side. B. Edge of the colony showing no firm outer sheath. C. Chain of spores. s. spore, gs. germinating spore, × 700. D. Vegetative trichome. h. heterocyst, vc. vegetative cell, sh. sheath.

trichome possessed a distinct wide gelatinous sheath (sh), which varied in width from 1.5-5μ. The vegetative cells (v.c.) of the filament measured from 4-5.5μ in diameter and from 6-9μ in length, in some cases the length and breadth being approximately equal. The heterocysts (h) were slightly larger, measuring 5-6μ in diameter and 8-10μ in length. The spores were oval, being 5-6.5μ in diameter and 8-9μ in length. Although these measurements are slightly larger than those given by Pascher for this species, the type of colony without any firm outer sheath, the presence of a wide sheath around each individual trichome, and the type of spores formed seem to place it without doubt as *N. paludosum*. This species seems to be mainly a freshwater form usually found in ditches and pools. This species has been recorded by Bailey(2) for Australia.

Nostoc muscorum Kütz.

(Figure 2.)

The occurrence of this alga in one flask only of the culture of garden soil A, and, contrary to the general rule, in a later and more dilute flask of the series—No. 16—showed that it was not widely distributed. The colonies formed flat, irregular masses which floated on the surface of the liquid medium among other algae in the flask. The filaments were flexuous and densely entangled. The vegetative cells measured 4-4.5μ in breadth and were nearly twice as long as broad (Fig. 2 A). Hetrocysts (h) were almost spherical, being approximately 5μ in diameter. The spores (Fig. 2 B) were longer than broad, approximately 6μ in diameter and 8μ in length, and were found in chains. The sheaths of the trichomes were for the most part invisible, being apparent occasionally towards the edges of the colonies.



Figs. 2-4.— 2. Nostoc muscorum Kütz. A. Vegetative trichome. B. Spores. 3. Anabaena minutissima Lemm. 4. Anabaena variabilis Kütz. h. heterocyst, s. spore, gs. germinating spore, vc. vegetative cell, ac. apical cell. × 700.

This species has frequently been recorded for the soil in other countries, but no previous record has been made for Australia.

Anabaena minutissima Lemm.

(Figure 3.)

Filaments which were found to correspond closely to the description of this alga were found growing among the algae in the cultures of garden soil A. The more or less straight filaments were isolated and did not form a definite colony. The vegetative cells measured 2-2.5μ in breadth and were slightly

shorter than broad, being 1.5-2μ in length. Spherical heterocysts, approximately 2μ in diameter, were present. A few elongated spores developed later and measured 18-20μ in length and 4-5μ in diameter.

Anabaena variabilis Kuetz.

(Figure 4.)

A second *Anabaena* species was found in the bush soil. The vegetative cells were approximately as long as broad, measuring 4.5-5μ in breadth and 4-5.5μ in length, the end cell being obtuse-conical. The heterocysts were intercalary, spherical or sometimes slightly longer than broad. Spores were later developed in chains and measured 7-8μ in diameter, and 9-13μ in length. They were never found next to the heterocysts.

Bristol-Roach(4) has recorded this species as present in English soils.

Phormidium foveolarum (Montague) Gom.

(Figure 5.)

This alga was found in garden soil A, always in association with *P. tenue* and *P. autumnale*, and was therefore very hard to separate and obtain in pure culture. The cells of the trichome were more or less quadrangular, sometimes shorter than broad, but never longer. The breadth measured approximately 1.5μ, while the length was from 0.8μ-1.5μ. The trichome was pale blue-green in colour and was distinctly constricted at the transverse walls. The end cell (a.c.) of the trichome was rounded and did not taper The sheath (sh) was narrow and colourless.

P. foveolarum is a terrestrial species, but has not previously been recorded for Australia.

Phormidium tenue (Menegh) Gom.

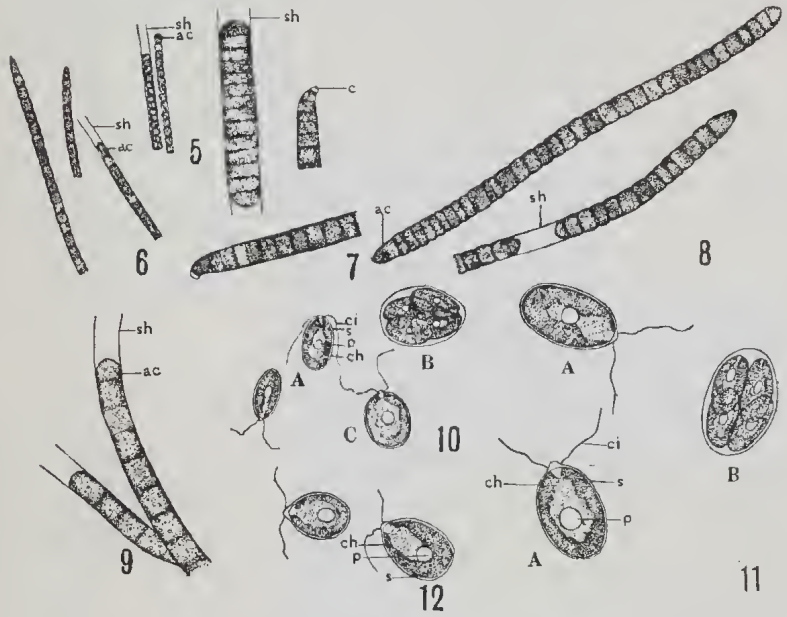
(Figure 6.)

In flasks Nos. 1 and 2 of garden soils both A and B, this species occurred frequently in association with *P. autumnale* and *P. foveolarum*, the three together forming a flat bluish-green matted mass adhering to the sides of the flasks or sometimes floating on the surface of the liquid.

Phormidium tenue is one of the two Myxophyceae which have been grown in pure culture on agar. The trichomes were a pale blue-green in colour, the cells measuring from 1.5-2μ in diameter and varying in length from 2-3μ, the end cell (a.c.) tapering. The trichome was slightly constricted at the transverse walls, which were more or less indistinct. The sheath (sh) was very narrow and could only be distinctly seen where the filament was broken and the sheath protruded past the trichome.

The species has a world-wide distribution in the soil, being recorded in England by Bristol Roach(4), in the United States of America by Moore and Carter(22), and in India by West.

It has also been recorded for Australia by Bailey(2).



Figs. 5-12.—5. Phormidium foveolarum Gom. 6. Phormidium tenue Gom. 7. Phormidium autumnale Gom. 8. Phormidium australe, n. sp. 9. Phormidium subterraneum n. sp. 10. Chlamydomonas communis Snow. A. Motile cell. B. Zoosporangium. C. Chlamydomonas communis var. ovata Playfair. 11. Chlamydomonas communis Snow var. grandis, n. var. A. Motile cell. B. Zoosporangium. 12. Chlamydomonas gracilis Snow. sh. sheath, ac. apical cell, c. calyptra, ci. cilia, s. stigma, p. pyrenoid, ch. chloroplast. × 700.

Phormidium autumnale (Ag.) Gom.

(Figure 7.)

This was another species which occurred in garden soil A in the first three flasks of the culture. The filaments were greyish-green in colour with very narrow sheaths (sh). The cells were shorter than broad, being 4.5-6μ in diameter and 3μ or less in length, and were not constricted at the transverse walls, which were often granulated. The filament tapered at the apex, which, as a rule, was slightly curved with a distinct calyptra (c) on the tip of the end cell. This species was often found in the flasks in association with *P. tenue* and *P. foveolarum,* but has not been obtained in pure culture.

This has been recorded for the soil in England by Bristol Roach(4).

PHORMIDIUM AUSTRALE, n.sp.

(Figure 8.)

A species of *Phormidium* occurred in flask No. 2 of the culture of garden soil A among the other species of this genus, but could not be identified with any form previously described. It was very slow in developing and did not appear in the flask in any appreciable quantity until after the other *Phormidium* species were more or less abundant. The trichome was a very light blue-green, the cells being approximately 4μ in breadth and slightly shorter than broad, with quite definite constrictions at transverse walls. The sheath was very narrow and was not apparent unless it protruded past the trichome. The end cell (a.c.) of the filament was elongated and tapering. *P. Jadinianum* Gom., which also measures approximately 4μ in the diameter of the cells, differs from this form in its colour, being an olive-green.

Cellulis palis caeruleis viridibus, latis 4μ, *latioribus paulo quam longioribus, constrictis ab muris transversis. Extrema cella villi extenta et attenuata.*

PHORMIDIUM SUBTERRANEUM, n.sp.

(Figure 9.)

In the cultures of bush soil another *Phormidium* was found which did not agree with any species previously described. The filaments were greyish in colour, with very indistinct transverse walls. The cells of the trichome were approximately 4.5μ in diameter and slightly longer than broad. The end cell (a.c.) was rounded, not tapering in any way and without a calyptra. The sheath (s) of the filament was narrow and showed only where it protruded past the trichome. It seemed closest to *P. ambiguum* Gom., which, however, is slightly constricted at the transverse walls.

Cellulis glaucis, latis 4-5μ, *longioribus paulo quam latioribus, non constrictis ab muris transversis quae confusa sunt. Extrema cella villi globosa, non extenta nec attenuata.*

Chlorophyceae.

These are plentiful in all cultures and the majority develop within a few weeks of inoculation. *Chlorococcum humicola* Rabenh. is the most frequent species, while *Stichococcus bacillaris* Näg. and a small species of *Chlorella* also appear constantly. Quite a number of these green forms have been obtained in pure culture on mineral salts agar. In the bush soil cultures, moss protonemas frequently occurred. The species identified are briefly described in the following pages.

CHLAMYDOMONAS COMMUNIS Snow.

(Figure 10.)

This species, although generally regarded as a plankton species, has been described from the soil by Bristol Roach(4) in England. In Victoria it was found in cultures from the bush soil and

occurred in many of the flasks. The cells were oblong-ovate and measured on an average 10-11μ in length and 5-6μ in diameter. At the anterior end of the cell there was a distinct papilla, from either side of which two long cilia arose. The chloroplast was cup-shaped with a distinct central pyrenoid. A lateral stigma occurred near the anterior end. Reproduction by division of the cell contents into daughter cells has been observed, the mother cell previously shedding its cilia and settling into a non-motile state (Fig. 10B).

Playfair (30) has recorded this species in New South Wales, and mentions also an ovate or broader form of this organism which he calls *C. communis* Snow var. *ovata*. This description seems to agree with a broader form of *C. communis* found in the soil cultures (Fig. 10c).

Chlamydomonas communis Snow var. grandis, n. var.

(Figure 11.)

This species also occurred in the cultures of the bush soil. It differed from the preceding species only in size, being very much larger, hence it was thought to be a new variety, *C. communis* var. *grandis*. The cells were oval with a distinct papilla at anterior end, from either side of which the two cilia arose. There was a cup-shaped chloroplast with a distinct central pyrenoid and a small lateral stigma near the anterior end of the cell.

Non-motile cells dividing to form daughter cells have been observed (Fig. 11B).

Chlamydomonas gracilis Snow.

(Figure 12.)

A third *Chlamydomonas* was found in the cultures from garden soil B. This species was broader in comparison with its length than the preceding species; also it tapered more towards the anterior end, being almost pear-shaped. The cell measured from 11-15μ in length and from 7-8μ in breadth. A papilla was present at the anterior end between the two cilia. The chloroplast was cup-shaped, with a large pyrenoid towards the posterior end of the cell. A lateral stigma was noted near the posterior end. Reproduction typical of the genus has been observed.

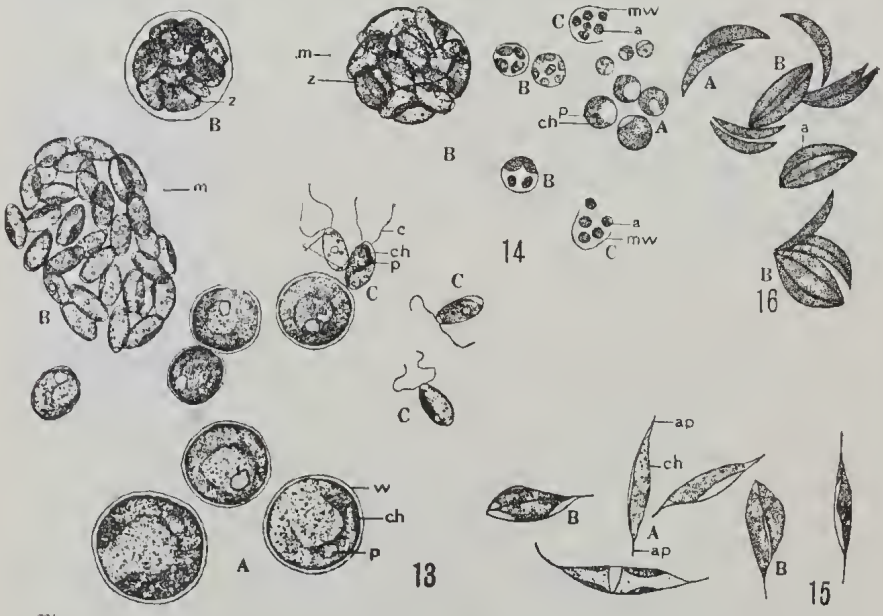
The measurements are slightly larger than those given by Pascher for this species, but otherwise it seems to agree closely with the description given.

Chlorococcum humicola (Naeg.) Rabenh.

(Figure 13.)

This is perhaps the most commonly occurring species, being found in most flasks of both garden and bush soil. It grows well on mineral salts agar and is obtainable in pure culture without much difficulty.

The vegetative cells (Fig. 13A) are round, bright green, and very granulated in appearance. The diameter of the cells varies, being on an average from 15-17μ, while the maximum is 22μ. There is a single parietal bell-shaped chloroplast (Fig. 13 ch), with one large pyrenoid (Fig. 13 p) which seems to lie opposite the opening of the chloroplast. The pyrenoid seems occasionally to divide into several parts. The cell wall (Fig. 13 w) is thin, about 0.5μ in thickness. Reproduction by zoospores (Fig. 13 z) is quite common. When the cell reaches a certain size the contents divide to form from 16-32 zoospores (Fig. 13B). When these are ready to be freed, the mother cell wall (w) becomes mucilaginous and finally bursts. The zoospores are approximately 9μ long and 5μ broad, with a single parietal chloroplast containing one pyrenoid. There is an unpigmented space (s) at each end of the zoospore. Two cilia (Fig. 13 c) of equal length (10-12μ) are attached to the basal end. The zoospores swim actively around and finally settle down, shed their cilia, and gradually become adult rounded vegetative cells.



Figs. 13-16.—13. Chlorococcum humicola Rabenh. A. Vegetative cells. B. Zoosporangia. C. Zoospores. 14. Chlorella vulgaris Beyerinck. A. Vegetative cells. B. Cells forming autospores. C. Autospores freed from mother cell. 15. Ourococcus bicaudatus Grobety. A. Vegetative cells. B. Cells dividing. 16. Ankistrodesmus falcatus Ralfs. A. Vegetative cells. B. Cells forming autospores, w. wall, ch. chloroplast, p. pyrenoid, m. mucilaginous wall, z. zoospores, ci. cilia, ch. chloroplast, a. autospore, mw. mother cell wall, ap. appendage. × 700.

This form agrees with the description given by Pascher, but seems slightly larger than the normal species. In this respect it agrees with the Malay form described by Bristol Roach(5).

This species has been recorded from the soil in England by Bristol Roach(4), and in America by Moore and Carter (22).

Chlorella vulgaris Beyerinck.

(Figure 14.)

This species also occurred frequently in the cultures of both bush and garden soil, and has been quite readily obtained in pure culture. The cells were round, varying in diameter from 5μ-9μ with a thin cell wall. There was a solitary parietal bell-shaped chloroplast (ch) with one pyrenoid (p) which was not always distinct.

The reproduction was by means of autospores, 4-8 of these being formed within the mother cell wall by the division of the cell contents, and being ultimately set free by the gelatinization of the old cell wall.

Several species of *Chlorella,* including *C. vulgaris,* have been recorded from the soil.

Ourococcus bicaudatus Grobéty.

(Figure 15.)

There seems to exist some doubt regarding the nomenclature of this species, synonyms being *Keratococcus caudatus* Pascher and *Dactylococcus bicaudatus* A. Br., but because of its ability to divide vegetatively it has been taken out of Oocystaceae; therefore, it cannot be a *Dactylococcus*, and the name *Ourococcus* seems to have priority over *Keratococcus*. This species was found in the cultures of bush soil only, in one or two of the less dilute flasks. It has been grown on plates of mineral salts agar, but as yet no very successful pure culture has been obtained.

The cells were fusiform in shape, straight or slightly bent with an appendage at both ends, both of which were pointed or one was pointed while the other was more or less rounded. The length of the cells measured from 25-34μ, including the appendages, which were approximately 5μ long. The diameter of the cells varied from 3.5-6μ. The chloroplast was peripheral, with one pyrenoid, which was often indistinct.

Reproduction by longitudinal division was observed (Fig. 15B). Although autospore formation may occur in this species, it has not been observed.

This species has been recorded for the soil by Bristol Roach(4) in England.

Ankistrodesmus falcatus Ralfs.

(Figure 16.)

This species agrees with the form described from the soil in England by Bristol Roach(4) which, because of its habitat and special resistant powers, she regarded as a special form of the species *A. falcatus* forma *terrestris*. It has been found in the cultures of bush soil only. The cells were crescent-shaped with pointed ends, and measured approximately 17-20μ in length and

2.5-3.5μ in breadth. A single parietal chloroplast covering the entire cell wall was present, but no pyrenoid has been observed. Reproduction was by longitudinal division into 4 to 8 autospores which are set free by the gelatinization of the mother cell wall.

Playfair(30) recorded the presence of two varieties of *A. falcatus* in plankton in New South Wales, while West(43) found it among the algae of the Yan Yean Reservoir, Victoria.

Oocystis solitaria var. terrestris, n. var.

(Figure 17.)

A species of *Oocystis* was grown in several cultures of garden soil A, being one of the first forms to develop, but dying out of the cultures completely within a few months.

The cells were always solitary, being elongated in shape and measuring approximately 7-9μ in diameter and 19-27μ in length. Each cell contained several chloroplasts without pyrenoids, and at both ends the wall was thickened slightly. Reproduction was by the formation of autospores, which measured on an average 6μ in diameter by 8-9μ in length. No mother cell with an unbroken wall was observed, but in many cases autospores were seen lying within portions of the old mother wall (see Fig. 17B).

The identification of this species was rather difficult. At first sight it closely resembled *O. rupestris* Kirchner, but upon closer observation was seen, unlike this species, to have polar thickenings of the cell wall. Pascher mentions several varieties of *O. solitaria* Wittrock. This form was very close to *O. solitaria* var. *elongata* Printz., from which, however, it differed in the type of polar thickening. It was therefore regarded as a new variety—*O. solitaria* var. *terrestris*.

Species of *Oocystis* are found as a rule in lakes and ponds, and as far as can be ascertained this is the first record of one from soil. West(43) has recorded *O. solitaria* Wittrock from the Yan Yean Reservoir, Victoria, and Playfair(29) has described many species and varieties of *Oocystis* in New South Wales, among them being *O. solitaria* Wittrock, all, however, from ponds or swamps.

Trochiscia hirta (Reinsch) Hansgirg.

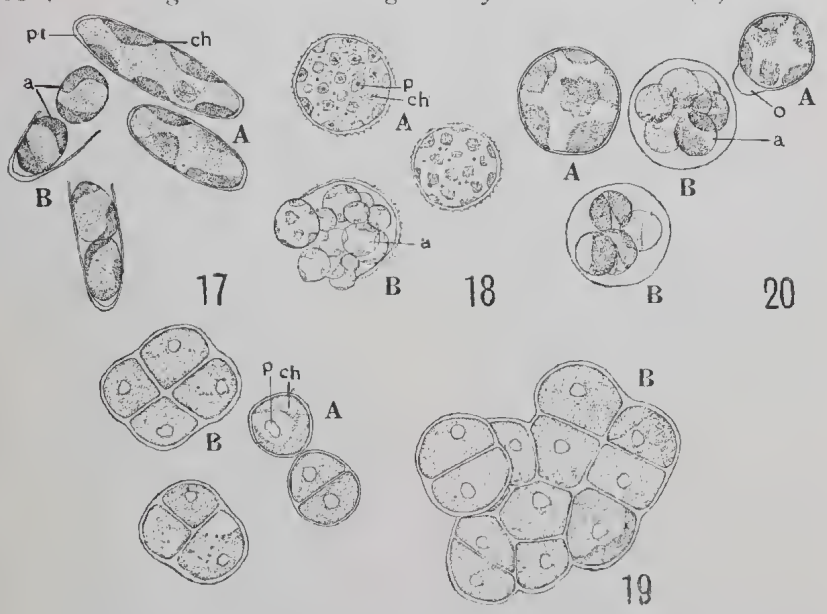
(Figure 18.)

This alga was found in several cultures of garden soils A and B, and has been obtained in pure culture. In this condition, unfortunately, the organism did not grow normally, and in many cases even developed an almost spineless wall. Some *Chorellas* have been described with appendages on their walls, but as this form contained several definite chloroplasts it seemed to be undoubtedly a *Trochiscia*. Following Pascher's key it was identified as *Trochiscia hirta*. With this identification, however, Professor Fritsch, to whom a culture of the organism was sent,

did not agree. However, it has been concluded that the culture, on reaching him, was not in a normal condition, probably even the spines on the walls were undeveloped or reduced.

The cells were spherical, varying in diameter from 9.5μ-21μ, with minute needle-like spines covering the outer wall (Fig. 18A). There were several chloroplasts (ch), slightly yellowish-green in colour, some with a pyrenoid (p). Reproduction by autospores was common (Fig. 18B), the spores developing the characteristic spines on their outer walls only after liberation from the mother cell.

Several species of *Trochiscia* have been recorded from the soil, including *T. hirta* in England by Bristol Roach(4).



Figs. 17-20.—17. Oocystis solitaria var. terrestris, n. var. A. Vegetative cells. B. Autospores freed from mother cell. 18. Trochiscia hirta Hansgirg. A. Vegetative cells. B. Autospores being freed from mother cell. 19. Protococcus viridis Agardh. A. Single cell. B. Colony. 20. Muriella australis, n. sp. A. Vegetative cell. B. Autosporangium. ch. chloroplast, pt. polar thickening, a. autospore, p. pyrenoid, o. outgrowth. × 700.

Protococcus viridis Agardh.

(Figure 19.)

Owing to one or two doubtful features, the identification of this species was left in abeyance for some time and the organism carefully observed in pure culture for over a period of two years. At the end of that time the organism was taken to be a *Protococcus* and more or less identical with *P. viridis*. This latter, however, usually has no pyrenoid, while in this form from the Victorian soil the pyrenoid was always very large and distinct (Fig. 19A (p)). West and Fritsch(44) draw attention to the fact

that there seem to be two species of *P. viridis,* one with a pyrenoid and one without. Whether that is so or not, it is certain that this species was never without one.

The cells were spherical, containing a single peripheral bright-green chloroplast (ch) with a large central pyrenoid (p). Adult cells measured on an average 9-10μ in diameter. Reproduction was always by vegetative division into two, then four, &c., often all the daughter cells remaining together to form a large colony (Fig. 19B, c). This is one of the commonest of the green algae, being world-wide in distribution. It is found as a green scum not only on damp soil, but on fences, tree-trunks, &c.

Muriella australis, n.sp.

(Figure 20.)

In many of the flasks of the cultures of garden soil B an organism was observed which could not be identified with any known species. It has been grown in pure culture, and carefully watched for over two years. The cells were solitary and spherical, and contained several green chloroplasts without pyrenoids. The chloroplasts were slightly yellowish-green in colour, but the cell walls gave a very definite cellulose reaction with chlor-zinc-iodine and no blue colour change when boiled with HCl, hence it could not be placed among the Heterokontae.

Many of the cells in an old culture developed peculiar outgrowths on the cell wall (Fig. 20A, o), which were not unlike those found in *Excentrosphaera.* However, these thickenings were by no means general.

Reproduction was by autospore formation, four or more being formed within the mother cell (Fig. 20B). Although this species has been grown in pure culture in mineral salts agar as well as in liquid mineral salts, no sign of zoospore formation has been observed.

The organism agreed with the generic characters given by J. Boye Petersen in his description of *Muriella,* but differed from his species in being much larger, measuring 10-18μ in diameter, also in having a thicker cell wall, and therefore the name *Muriella australis* is proposed for it.

Cellulis globosis, latis 10-18μ *cum muris modice crassis, et aliquot irridibus chromatophoris sine pyrenoidibus. Reproductione per autospores.*

Ulothrix variabilis Kützing.

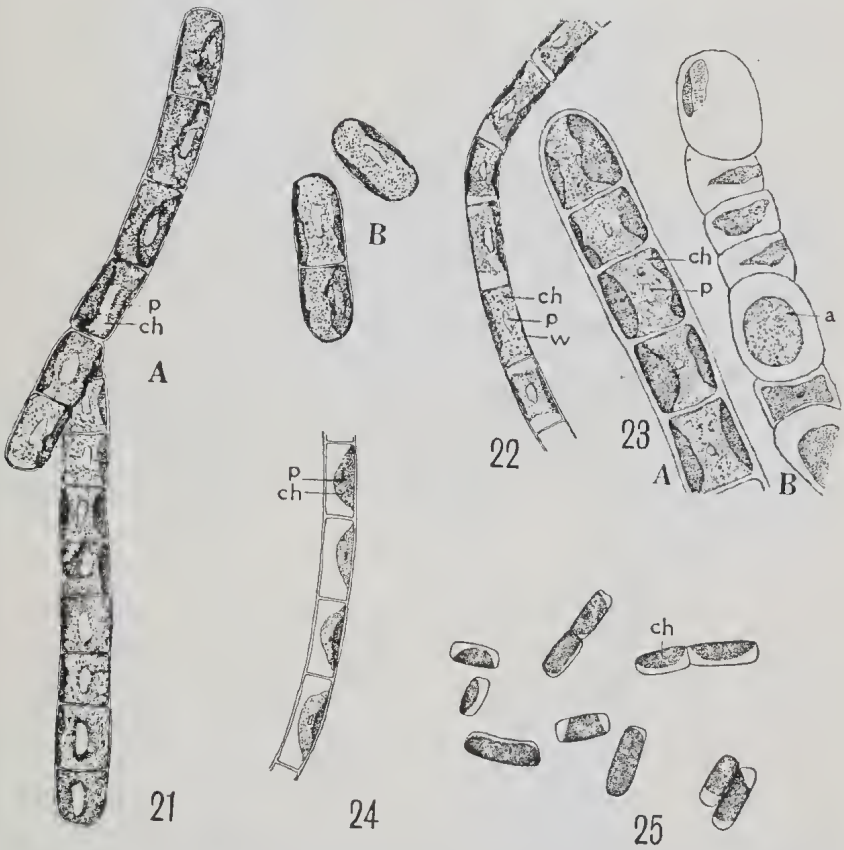
(Figure 21.)

U. variabilis Kütz. was fairly common in the cultures from garden soils A and B, but was never found in the cultures of bush soil. It has been obtained in pure culture without any difficulty.

The organism consisted of long unbranched filaments, the individual cells of which measured 12-13μ in length and 7-8μ in diameter, with a single green chloroplast (ch) encircling

two-thirds of the protoplast, and containing one large central pyrenoid (p). The end cell of the filament was rounded at the apex. In a fairly old culture the filaments break up into 2 or 3-celled fragments (Fig. 21B). This vegetative type of reproduction was the only kind observed, the typical method by zoospore formation not taking place in the cultures.

This species has been described by Bristol Roach(4) for the soil in Great Britain.



Figs. 21-25.—21. Ulothrix variabilis Kütz. A. Vegetative filaments. B. Filaments breaking up. 22. Ulothrix subtilissima Rabenh. 23. Ulothrix aequalis Kütz. A. Vegetative filament. B. Filament forming akinetes. 24. Hormidium flaccidum A. Br. 25. Stichococcus bacillaris Nag. p. pyrenoid, ch. chloroplast, w. thin cell wall, a. akinete. × 700.

Ulothrix subtilissima Rabenhorst.

(Figure 22.)

Unlike the preceding one, this species was found in the bush soil only, and did not appear in any of the flasks containing garden soil. It has not yet been obtained in pure culture. The filaments were narrower than those of *U. variabilis,* measuring from 4.5-5.5μ in diameter, while the individual cells varied in

length from 11-13μ, and contained a single chloroplast (Fig. 22 ch), encircling two-thirds of the protoplast with one central pyrenoid (p). The cell wall (w) was comparatively thin.

In this species of *Ulothrix* again no zoospore formation could be observed. This species does not appear to have been previously recorded for the soil.

Ulothrix aequalis Kützing.

(Figure 23.)

This form was observed in cultures of the bush soil, and although small colonies have been found growing on agar plates, no pure culture has yet been obtained.

The thick cell wall distinguished it from the foregoing species of *Ulothrix*. The cells of the filaments measured approximately 12μ in diameter, and were as long as broad, sometimes slightly longer. Each cell contained a single chloroplast (ch), which covered the entire longitudinal wall and encircled two-thirds of the protoplast. According to Pascher there may be two pyrenoids present in each cell, but in all these cultures each cell contained one pyrenoid only (p).

No zoospore formation has been observed, but in many filaments, particularly those in older cultures, the contents of the cell have rounded off to form akinetes, the longitudinal walls of the filament in these cases being constricted (Fig. 23B).

This species does not appear to have been previously recorded from the soil.

Hormidium flaccidum A. Br.

(Figure 24.)

A few filaments which have been identified as *Hormidium flaccidum* A. Br. were found growing in one or two of the earlier or less dilute flasks of bush soil. The size of the chloroplast, which covered a small portion only of the cell wall, distinguished this genus from *Ulothrix*. The filaments were long and unbranched and did not break up as readily as many species of *Hormidium*. The individual cells of the filaments measured from 5-6μ in diameter and from 11-15μ in length. The chloroplast (ch) did not cover more than half of the cell wall, and contained a distinct pyrenoid (p). The vegetative filaments only have been observed, no reproduction having taken place.

Bristol Roach(4) records the presence of *H. nitens* Menegh, but this species does not appear have been previously recorded.

Stichococcus bacillaris Naegelli.

(Figure 25.)

This species occurred frequently in the cultures from both bush and garden soil. It grew particularly well on the mineral salts agar, and formed quite a large colony in a few weeks and was comparatively easy to obtain in pure culture. Although it

belongs to the Ulotrichales, the filamentous form was rarely seen in culture, single cells predominating or two or three cells united in short chains.

The cells agreed in all particulars with the typical form as described by Pascher, being from 3-3.5μ in breadth and varying in length from 6-11μ, the ends being rounded. There was a single plate-like chloroplast (Fig. 25 c), which filled about two-thirds of the cell, but no pyrenoid was present.

This species is a common terrestrial form and has been recorded for the soil by Bristol Roach(4) in England and by Moore and Carter in Missouri, United States of America(22).

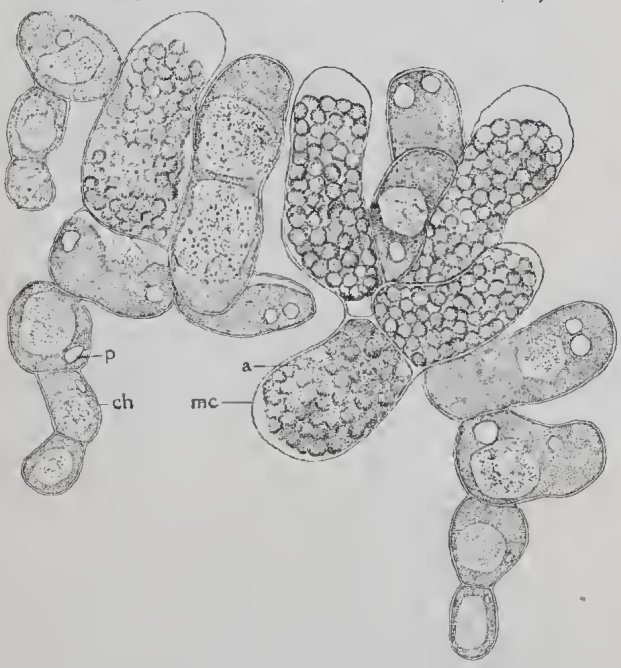


Fig. 26. Gongrosira australis, n. sp. ch. chloroplast, p. pyrenoid, mc. mother cell, a. spore.

GONGROSIRA AUSTRALIS, n. sp.

(Figure 26.)

This organism occurred frequently in the cultures of bush soil, either as small irregular colonies or short filaments two or three cells in length. It has been grown on mineral salts agar, where it formed spherical colonies. By its vegetative characters it was identified as a *Gongrosira*, but further investigation showed that no zoospores were ever formed. The organism was carefully watched for over a year, and during that period many of the larger cells of the filaments became gonidangia, their contents dividing up to form numerous small round gonidia or autospores which were never motile.

However, it was concluded that this was due to cultural conditions, as neither *Botrydiopsis arhiza* Borzi nor *Heterococcus viridis* Chodat, both of which normally form zoospores, did so under conditions identical with those under which this alga was grown. It was, therefore, decided that this species belonged to *Gongrosira,* and it is suggested that the generic description be modified to include the formation of non-motile spores under cultural conditions. The species, however, did not agree with any previously described, being closest to *G. terricola* Bristol but larger in size with both intercalary and terminal gonidangiums.

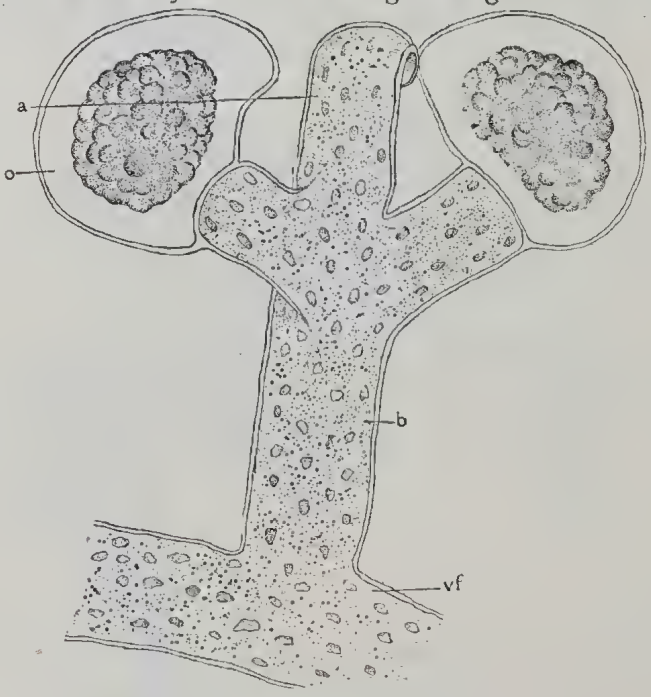


Fig. 27. Vaucheria hamata (Vauch.) Lyngb. × 400. vf. vegetative filament, b. sexual branch, o. oogonium, a. antheridium.

The thallus consisted of cells of varying sizes branching irregularly to form colonies, only slightly differentiated into basal cushion-forming cells and upper filamentous ones, the end cells of the filament gradually decreasing in size. There was a single irregular chloroplast (ch) in each cell, usually with one pyrenoid (p) but occasionally with two or even three. The vegetative cells were generally longer than broad, measuring from 8-15μ in diameter and from 12-45μ in length. Any of the larger cells in the filament was apparently capable of becoming a gonidangium (Fig. 26 mc), the contents dividing up to form numerous small round gonidia or autospores (a). The wall at the apex of the mother cell became swollen and mucilaginous, finally breaking down to free the gonidia.

Filamentis inferioribus cum cellulis subglobosis et superioribus erectis cellulis 8-15μ *latis, et longis* 12-45μ *cum singularis inaequalibus chromatophoris et* 1-3 *pyrenoidibus. Reproductione per gonidangias extremas vel intercalarias cum gonidiis multis rotundis.*

VAUCHERIA HAMATA (Vauch.) Lyngb.

(Figure 27.)

This alga did not grow in any of the culture flasks but grew in abundance on the surface of a sample of the garden soil A which had been placed in a sterile petri dish. The filaments (vf) measured approximately 35-40μ in diameter. Oogonia (o) were found on lateral branches, either one or two being present on each branch from which an antheridium (a) also grew. An oogonium measured about 70μ x 56μ, being subspherical, while an antheridium was 26μ in diameter, and very much curved. This species is frequently found growing on damp soil.

Heterokontae.

Only five species of Heterokontae have been identified from Victorian soils, and of these *Bumilleria exilis* Klebs is the most frequent form, occurring in garden soil A and B as well as in the bush soil. *Heterococcus viridis* Chodat occurs in one series of the garden soil and in the bush soil. *Botrydiopsis arhiza* Borzi also is a very common terrestrial form, being world-wide in its distribution, but here it was found only in one series of the garden soil cultures.

Brief descriptions of the five species are given.

OPHIOCYTIUM TERRESTRE Heyward.

In a previous paper(18) the author has described this species, which was found in the cultures of bush soil. The organism grew readily in the flasks but could not be obtained in pure culture. It was a non-colonial form not attached in any way to some substratum. The size of the adult cell (A) varied from 9-12μ in breadth and up to 135μ in length. The cell was slightly curved and had a distinct apical cap (a.c.), while at the basal end was a wavy stalk. Reproduction by both aplanospores and zoospores was observed.

This was the first occasion on which the genus was recorded from soil.

BOTRYDIOPSIS ARHIZA Borzi.

(Figure 28.)

This species was identified from cultures of garden soil B, but has not been obtained in pure culture. The cells were solitary and large, with very thin walls, and contained numerous yellowish-green disk-like chloroplasts. The cells varied in diameter from 15-30μ, the smaller ones containing fewer chloroplasts.

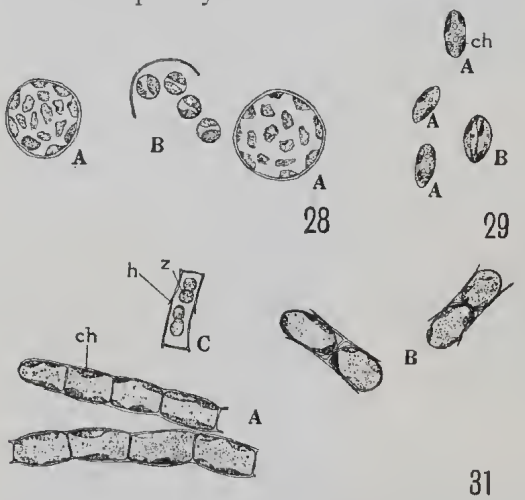
No zoospores were observed, but autospore formation was frequent, this being probably due to cultural conditions. The contents of the mother cell divided to form numerous rounded spores which were freed by the bursting of the mother cell wall (Fig. 28B).

This species is a very common terrestrial alga and has frequently been recorded for the soil.

CHLOROCLOSTER MINOR, n. sp.

(Figure 29.)

A small brownish-green unicellular alga was frequently observed in the earlier stages of development of the cultures of bush soil. Unfortunately, it could not be obtained in pure culture and later died out completely.



Figs. 28, 29, 31.—28. Botrydiopsis arhiza Borzi. A. Vegetative cell. B. Autospores being freed from mother cell. 29. Chlorocloster minor n. sp. A. Vegetative cell. B. Autosporangium. 31. Bumilleria exilis Klebs. A. Vegetative filaments. B. Filaments breaking down to form akinetes. C. Cell forming zoospores. ch. chloroplast, s. autospores forming, z. zoospores, h. h-shaped pieces showing. × 700.

The cells were solitary, elongated in shape, and contained two or more brownish-green chloroplasts. No starch reaction was given with iodine, while with chlor-zinc-iodine the cell walls gave no cellulose reaction. The alga was therefore placed in the Heterokontae and was found to be very similar to *Chlorocloster terrestris* Pascher, from which it differed in size, being much smaller. The length measured from 7-8μ while the diameter was 2.5-4μ. The cell wall was very thin, and each cell contained two or more chloroplasts (ch).

Reproduction took place by longitudinal division into autospores (Fig. 29B).

Cellulis solitariis extentis latis 2.5-4μ, *longis* 7-8μ *cum duo aut multis chromatophoris fuscis irridibus. Reproductione per autospores.*

Heterococcus viridis Chodat.

(Figure 30.)

This alga grew in the cultures of bush soil and garden soil A. It developed in a few weeks after inoculation and in the earlier examinations was one of the most prominent forms. Later it apparently died out of the culture. This disappearance, however, may have been due to the fact that the more mature filaments tended to break down into unicellular spherical organisms. This was proved by growing a colony on mineral salts agar and watching its development (see Fig. 30A, B, C).

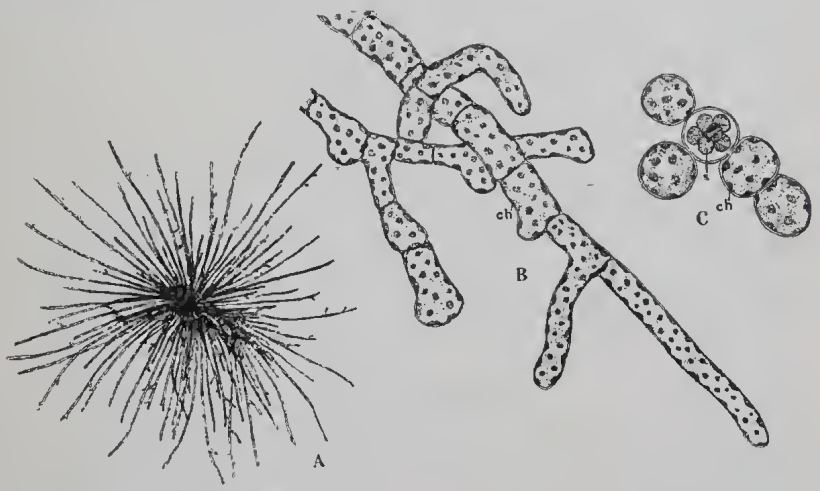


Fig. 30. Heterococcus viridis Chodat. A. Young colony. B. Portion of filament. C. Filament breaking up into unicells. ch. chloroplast, s. autospores forming. × 700.

In its filamentous form, the organism was built up of cells of irregular shape and size, longer than broad, and containing numerous disk-like yellowish-green chloroplasts (ch). No pyrenoids were present and the cell walls gave a negative chlorophyll test with chlor-zinc-iodine. The diameter of the cells averaged from 7-10μ, while the length varied from 10-60μ. In the unicellular form the diameter measured from 10-15μ, and in many of these cells the contents divided up to form numerous small spores (Fig. 30c (s)). These may have been autospores or zoospores which had not then become motile, both types of reproduction taking place. This form has been recorded for the soil by Moore and Carter (22) in America.

BUMILLERIA EXILIS Klebs.

(Figure 31.)

This was perhaps the most frequently occuring member of the Heterokontae, being present in numerous flasks of both bush and garden soil. Identification of this species was difficult because the so-called characteristic H-shaped pieces in the cell wall were not very evident in the vegetative condition. This is apparently always the case in this species of the genus, and on this account Pascher suggests taking it out of *Bumilleria* and renaming it *Heterothrix exilis*.

The organism consisted of comparatively short unbranched filaments, the individual cells measuring 4-5μ in diameter and 10-15μ in length. In each cell there were usually two chloroplasts (ch), but occasionally more. The species has been obtained in pure culture on mineral salts agar and under these conditions reproduction took place by the liberation of akinetes. The contents of an individual cell rounded off to form a spherical spore which is set free by the breaking of the cell wall (Fig. 31c). In these circumstances H-shaped pieces are more apparent. In one instance only was more than one spore in a cell observed. Here the contents rounded off to form four small spores. It was impossible to tell whether these were zoospores or autospores as no motile organs were observed (Fig. 31B).

This species has been previously recorded for the soil by Bristol Roach (4) in England.

Bacillariaceae.

Very few diatoms or desmids have been identified in the culture flasks. They do not appear until long after the other algae are well established, and only two have been identified with certainty.

HANTZSCHIA AMPHIOXYS (Ehr.) Grun.

(Figure 32.)

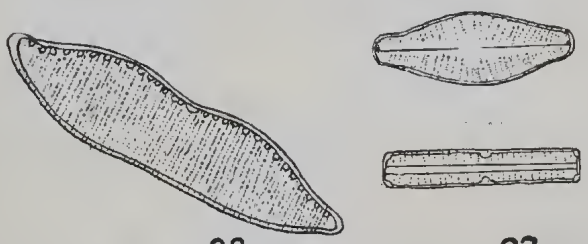
This was the only diatom which was found in any appreciable quantity. It measured on an average 7μ x 33μ. This form has been described by Bristol Roach (4) from the soil in England and by Moore and Carter (22) in U.S.A.

NAVICULA MUTICA Kütz.

(Figure 33.)

This diatom was identified in many flasks of the cultures from garden soils A and B. The breadth of the valve averaged 6μ while the length varied from 14-17μ. This species was found by Bristol Roach (4) to be one of the commonest soil diatoms.

From this investigation, it will be realized how very cosmopolitan are the terrestrial algae in their distribution, many forms identified from soils in England, America, and Europe, reappearing here in Victorian soils. It is very significant to find three algae—*Chlorococcum humicola, Bumilleria exilis* and *Hantzschia amphioxys,* which Bristol Roach (4) points out as being three of the most frequently occurring species in English soils—again occupying that position in regard to Victorian soils. Quite a number of other species which have been identified in other parts of the world are also present here, including *Nostoc muscorum, Anabaena variabilis, Phormidium tenue,*



Figs. 32. Hantzschia amphioxys (Ehr.) Grun. 33. Navicula mutica Kütz. × 700.

Phormidium autumnale, Chlamydomonas communis, Chlorella vulgaris, Ourococcus bicaudatus, Ankistrodesmus falcatus, Trochiscia hirta, Stichococcus bacillaris, Vaucheria hamata, Botrydiopsis arhiza, and *Heterococcus viridis.* Some few, such as *Oocystis solitaria, Ulothrix subtilissima, Ophiocytium terrestre,* have not previously been recorded from the soil, while others again were found to be new species belonging, for the most part, to terrestrial genera e.g., *Phormidium australe, Phormidium subterraneum, Muriella australis, Gongrosira australis, Chlorocloster minor.*

In conclusion I should like to thank all those who have helped me during this investigation—Professor Ewart for his unfailing interest, Dr. McLennan for her ever willing assistance and helpful suggestions, and Professor Fritsch of the London University, to whom many specimens were forwarded for verification.

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